Peer Review

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HEALTH EFFECTS DIVISION
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EPA SERIES 361

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Health Effects Division (HED) Peer Review Committee
Draft Document on MALATHION

FROM: Esther Rinde, Ph.D. E.R.
Manager, HED Carcinogenicity Peer Review
Science Analysis Coordination Branch
Health Effects Division (H7509C)

TO: Addressees

Attached for your review is the draft document of the Peer Review Committee on MALATHION prepared by Dr. Kerry L. Dearfield. Please provide your comments on the draft document and return to me no later than February 26, 1990. If a reply is not received by that time, we will presume that you concur and have no comments.

Should you need a few extra days for a thorough review, please let us know that your comments are forthcoming.

ADDRESSEES

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MEMORANDUM

SUBJECT: DRAFT Peer Review of Malathion

FROM: Kerry L. Dearfield, Ph.D.
Executive Secretary, Peer Review Committee
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Joanne Edwards
Review Manager
Special Review and Reregistration Division (H7508C)

The Health Effects Division Peer Review Committee met on February 7, 1990 to discuss and evaluate the weight-of-the-evidence on Malathion with particular reference to its carcinogenic potential. The Committee unanimously agreed to classify malathion as a Group D Carcinogen; that is, malathion is not classifiable as to human carcinogenicity. This decision was based on the inadequacy of the available studies to make a definitive determination of the carcinogenicity of malathion. The Committee reaffirmed the requirements of the Malathion Reregistration Standard that requires the Registrant to perform an additional mouse carcinogenicity study with malathion and an additional rat carcinogenicity study with malaoxon. The Committee also determined that the Reregistration Standard recommendation to perform a carcinogenicity study in combination with a rat chronic study on malathion be made into a requirement.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Penelope A. Fenner-Crisp	_____
William L. Burnam	_____
Karl Baetcke	_____
Marcia Van Gemert	_____
John Quest	_____
Esther Rinde	_____
Kerry Dearfield	_____
Richard Levy	_____

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Marion Copley _____
George Ghali _____
Richard Hill _____
Robert Beliles _____
Julie Du _____
Yin-Tak Woo _____

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Brian Dementi _____
Roger Gardner _____

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Reto Engler _____

4. Other Attendees: Bruce Jaeger, HED
Bernice Fisher, HED
Hugh Pettigrew, HED
Linda Kutney, HED

B. Material Reviewed:

The material available for review consisted of 1) a draft Toxicology Branch I response to the Registrant's technical response to the Malathion Reregistration Standard; 2) selected pages from the Malathion Reregistration Standard (issued February, 1988); 3) reviews of carcinogenicity studies on malathion and malaoxon (consisting of memoranda and DER's); 4) journal publication Huff et al. (Environ. Res. 37: 154-173, 1985) on the National Toxicology Program (NTP) reevaluation of malathion and malaoxon National Cancer Institute (NCI) rat carcinogenicity studies; 5) memorandum of E. McConnell to J. Moore, June 14, 1984, status report on NTP review of NCI malathion carcinogenicity studies; and 6) memorandum from A. Gross (April 24, 1984) concerning the carcinogenicity of malathion.

This package was prepared by Dr. Brian Dementi of Toxicology Branch I, Health Effects Division. The discussion on each of the individual carcinogenicity studies follows the presentations by Dr. Dementi. The material reviewed is attached to the file copy of this report.

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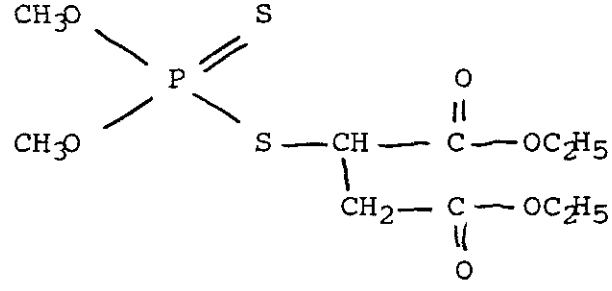
C. Background Information: A chemical name for malathion is S-[1,2-bis(ethoxycarbonyl)ethyl]-O,O-dimethyl phosphorodithioate. It is also known as Cythion among many synonyms. Malathion is an organophosphate insecticide and miticide. It is used on a wide variety of food and non-food crops as well as for insect control for both outdoor and indoor situations. Its primary mode of activity is through cholinesterase inhibition. There are several basic producers of malathion in the United States.

The Chemical Abstracts Service (CAS) Registry number for malathion is 121-75-5 and the Tox Chem Number (or Caswell number) is 535. The CAS Registry number for malaoxon (the oxygen analog of malathion; the double bonded S in malathion is replaced by a double bonded O in malaoxon) is 1634-78-2.

Structure of Malathion:



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D. Evaluation of Carcinogenicity Evidence for Malathion:

There were four carcinogenicity studies reviewed using malathion as the test chemical (total of three rat studies using Osborne-Mendel, Fischer 344 and Sprague-Dawley rats and one in B6C3F1 mice). There were two carcinogenicity studies reviewed using malaoxon as the test chemical (one in Fischer 344 rats and one in B6C3F1 mice).

1. Malathion - Osborne-Mendel Rat Dietary Feeding Carcinogenicity Study

Reference: National Cancer Institute. 1978. Bioassay of malathion for possible carcinogenicity, CAS No. 121-75-5. Technical Report Series, No. 24, National Cancer Institute, Bethesda, MD. NCI-CG-TR-24. Assay performed at Gulf South Research Institute, New Iberia, LA. Report authored by M. Steinberg et al. at Tracor Jitco under NCI direction. Reviewed in Document # 000314.

Malathion (technical grade; purity ≥95%) was administered in the diet to groups of 50 male and 50 female Osborne-Mendel rats (from Battelle Memorial Institute, OH) at time weighted average dosage levels of 4700 or 8150 ppm per group for 80 weeks. Animals were then observed for an additional 29 to 33 weeks. Low dose animals received 8000 ppm in the diet for an initial 14 weeks, which was then adjusted to 4000 ppm for the remaining 66 weeks. High dose animals received 12,000 ppm for an initial 3 weeks, which was then adjusted to 8000 ppm for the remaining 77 weeks. Matched controls consisted of groups of 15 untreated rats of each sex (however, it is noted that matched controls are reported as 2 groups of 10 animals/sex for low dose matched controls and 5 animals/sex for high dose matched controls; the reason for this was that there was an abortive start to the high dose group and when it was reinitiated, the 5 high dose matched controls per sex were added to the original 10 matched controls per sex). Pooled controls consisted of the matched controls combined with 40 untreated male and 40 untreated female rats from similar bioassays of four other test chemicals. These other pooled controls came from bioassays performed at the same laboratory and overlapped the malathion bioassay by at least 1 year. All surviving rats were killed at 108 to 113 weeks.

The Peer Review Committee decided that the analyses that should be of primary importance would be the NTP reevaluation of the original NCI studies (this applies to the three NCI rat studies, two with malathion and one with malaoxon) (these analyses are found in the Huff et al., 1985 journal article and the E. McConnell memorandum to J. Moore). The Committee felt that the NTP reevaluation provided a more extensive evaluation and there was a consensus of opinion on the examined tumors by a panel of pathology experts.

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a. Discussion of Tumor Data

The original NCI review indicated a statistically significant dose-related trend for follicular cell carcinomas/adenomas/ hyperplasia of the thyroid in female rats. However, there was no significance from a pair-wise comparison. The NCI concluded "there was no clear evidence of the association of the tumor incidence with the administration of malathion."

The NTP reevaluated tissues from these organs (thyroid gland as well as adrenal gland) as potential suggestive targets. They reaffirmed the original NCI conclusion by stating "under the conditions of these studies, there was no evidence of carcinogenicity in male or female Osborne-Mendel rats that received time-weighted average doses of 4700 or 8150 ppm malathion in their diet for 80 weeks." NTP examinations of the major sites of potential targets are shown in Tables 1 and 2. In particular, the NTP reevaluation diagnosed additional follicular cell adenomas in the control and low dose groups that eliminated the positive trend the NCI reported. It was noted by the Peer Review Committee that the NTP did not report hyperplasia incidence although the NTP states in the McConnell memo that there was no hyperplasia effect (this may be important as the development of thyroid tumors may be part of a progression from hyperplasia to adenoma to carcinoma).

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Table 1: Malathion Osborne-Mendel Rat Study - Thyroid Findings of NTP Reevaluation

	Sex	C1	C2	Low Dose	High Dose
No. Tissues Examined	M	14	41	35	40
	F	14	41	44	42
C-cell adenoma and carcinoma	M	1 (7.1%)	3 (7.3%)	1 (2.9%)	7 (17.5%)
	F	2 (14.3%)	10 (24.4%)	2 (4.5%)	5 (11.9%)
Follicular Cell Adenoma & Carcinoma	M	2 (14.3%)	8 (19.5%)	9 (25.7%)	12 (30.0%)
	F	0 (0%)	1 (2.4%)	1 (2.3%)	4 (9.5%)

C1 = Matched Controls
C2 = Pooled Controls
(Incidence %)

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Table 2: Malathion Osborne-Mendel Rat Study - Pheochromocytoma Findings of NTP Reevaluation

Males	C1	C2	Low Dose	High Dose
Adrenal Gland Pheochromocytoma	0/14 (0%)	2/50 (4%)	0/46 (0%)	5/44 (11%)

C1 = Matched Controls
C2 = Pooled Controls
Number of Lesions/Number of tissues examined (Incidence %)

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The Reregistration Standard for malathion states "the Agency agrees with the NCI/NTP that malathion is not carcinogenic in Osborne-Mendel rats." However, subsequent considerations by Toxicology Branch I/Health Effects Division and this Peer Review Committee raise doubt as to how definitive this study is to make a clear conclusion about the carcinogenicity of malathion. From the NTP reevaluation, there is not enough data presented (e.g. individual animal data, information about the "pooled" controls) to allow an independent statistical treatment of the data by statisticians supporting the Peer Review Committee. This makes it difficult for the Peer Review Committee to make an independent decision based on their own analyses of the data. Therefore, it is not entirely clear that the suggestive evidence of male C-cell adenomas/carcinomas, of male and female follicular cell adenomas/carcinomas, and of male adrenal pheochromocytomas can be totally dismissed.

b. Considerations of Study Adequacy for Assessment of Carcinogenic Potential

Other considerations provide even greater doubt about the adequacy of this study to make definitive conclusions regarding the carcinogenicity of malathion. 1) This study was performed before the Good Laboratory Practice (GLP) standards were in place. A significant deviation from current GLPs is the length of dosing in this study where rats received malathion for 80 weeks instead of a total 2 year period. 2) The number of concurrent control animals (15) is a very small group; this makes comparative analyses difficult when there is not a dramatic difference between tumor incidences. In addition, the concurrent "matched" control group was divided into two groups of 10 and 5 rats each. These two subgroups exhibited different weight gains, which serve to further confound comparative treatment of the data. Furthermore, it cannot be concluded with certainty that if a larger concurrent control size had been used, the incidence of spontaneous tumors would rise proportionately. 3) Without detailed information about the pooled control animals, the appropriateness of using these animals as "concurrent" controls is unclear. It was also noted in this regard

that the historical control incidence from this laboratory was not available to the Peer Review Committee during these deliberations. 4) The NTP states that malathion had no significant effect on survival of male and female Osborne-Mendel rats. However, there were suggestions of a dose-related decrease in survival for both male and female animals near the end of the study. This may provide doubt about the actual number of animals at risk for tumor induction. The issue of whether life-table analysis would be appropriate or not was raised. Without detailed animal data, this could not be resolved.

2. Malathion - Fischer 344 Rat Dietary Feeding Carcinogenicity Study

Reference: National Cancer Institute. 1979. Bioassay of malathion for possible carcinogenicity, CAS No. 121-75-5. Technical Report Series, No. 192, National Cancer Institute, Bethesda, MD. NCI-CG-TR-192. Assay performed at Gulf South Research Institute, New Iberia, LA. Report authored by C.R. Angel et al. at Tracor Jitco under NCI direction. Reviewed in Document # 000314.

Malathion (manufacturer's assay; purity 95%) was administered in the diet to groups of 49 to 50 Fischer 344 rats of each sex (from NCI Frederick Cancer Research Center, MD) at doses of 2000 or 4000 ppm per group for 103 weeks. Animals were then observed for an additional 2 or 3 weeks. Matched controls consisted of 50 untreated rats per sex. All surviving rats were killed at 105 to 106 weeks.

a. Discussion of Tumor Data

The original NCI review stated "malathion was not carcinogenic in male or female rats, but the females may not have received a maximum tolerated dose." The NCI acknowledged that the increase in adrenal gland pheochromocytoma at low dose males was statistically significant by pair-wise comparison, but did not consider this to be associated with the administration of malathion. This conclusion was based on the lack of an effect at the high dose and the lack of a dose response effect.

The NTP reevaluated tissues from the male adrenal gland as a potential suggestive target. They reaffirmed the original NCI conclusion by stating "under the conditions of these studies, there was no evidence of carcinogenicity in male or female Fischer 344 rats that were provided diets containing 2000 or 4000 ppm malathion for 103 weeks." The NTP suggests that two neoplasms appeared to be increased in the low dose males, pheochromocytoma of the adrenal gland (Table 3) and leukemia (Table 4; note the NCI did not comment on this lesion). However, these marginal increases were only significant by life-table analyses. The NTP suggests that life-table analyses are appropriate if the lesion is the cause of death.

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The NTP judges that the early deaths seen in this study (discussed below) is due to chemical toxicity. However, the increases in these two tumor types were not significant by incidental tumor tests or pair-wise tests and malathion was concluded not to provide evidence of carcinogenicity. The NTP suggests that the reduced survival in the dosed groups made the overall interpretation of these data difficult.

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Table 3: Malathion Fischer 344 Rat Study - Pheochromocytoma Findings of NTP Reevaluation

Males	Control	Low Dose	High Dose
Adrenal Gland Pheochromocytoma	5/49 (10%)	10/48 (21%)	6/46 (13%)

Number of Lesions/Number of tissues examined (Incidence %)

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Table 4: Malathion Fischer 344 Rat Study - Leukemia Findings of NTP Reevaluation

Males	Control	Low Dose	High Dose
Hematopoietic System - Leukemia	13/50 (26%)	20/50 (40%)	8/49 (16%)

Number of Lesions/Number of tissues examined (Incidence %)

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The Reregistration Standard for malathion states "the Agency agrees with the conclusions of the NCI/NTP, but notes that the dose levels employed in this study were approximately one-half of those employed in the NCI Osborne-Mendel rat study, and that therefore it is unlikely the maximum tolerated dose was reached in females." However, subsequent considerations by Toxicology Branch I/Health Effects Division and this Peer Review Committee raise doubt as to how definitive this study is to make a clear conclusion about the carcinogenicity of malathion. Again, as in the Osborne-Mendel rat study, the NTP does not provide detailed data from which to perform independent statistical analyses. The large decrease in survival of exposed males confounds the interpretation of potential tumor induction. Therefore, it is not entirely clear that the suggestive evidence of male adrenal pheochromocytomas and of male leukemia at the low dose can be totally dismissed.

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b. Consideration of Study Adequacy for Assessment of Carcinogenic Potential

Other considerations provide even greater doubt about the adequacy of this study to make definitive conclusions regarding the carcinogenicity of malathion. 1) Like the Osborne-Mendel rat study, this study was performed before the Good Laboratory Practice (GLP) standards were in place. However, unlike the Osborne-Mendel rat study, this study employed a more appropriate number of concurrent control animals and dosing was carried out over the 2 year period. 2) While this study employed lower doses than the Osborne-Mendel rat study, there was a major problem with mortality in the Fischer 344 rats, especially in the males. Survival at 103 weeks for males was 54%, 28% and 0% for control, low- and high-dose groups, respectively. For females, the comparable figures were 64%, 52% and 50%. In males, it was noted there was not a great disparity in survival figures after 90 weeks, so increased mortality rates appeared after this time. The large drop in survival in males confounds the observations found at the high dose where apparently less animals were at risk for tumor induction. It is not clear how this may have impacted upon a possible dose-response association. Despite the small decrease in survival for females, it was suggested that the top dose was not high enough for a definitive assessment of carcinogenic potential. 3) The concurrent control incidence for pheochromocytomas in males tabulated by the NTP was closely examined. In their reevaluation, this incidence is reported to be 10% (5/49 animals). The original NCI review reported the incidence to be 4% (2/49 animals; three less than the NTP reevaluation). This latter value appears more in line with the historical control incidence from the testing laboratory of 3% (8/275 animals) among males. It is not known what the range of control values from separate studies in the historical database is from the testing laboratory. Therefore, the issue of the NTP concurrent control incidence and what it means to the statistical evaluation of the tumors observed in this study is not resolved.

c. Non-Neoplastic Findings

There were several significant non-neoplastic findings that deserve mention. Stomach inflammation and ulceration were clearly increased in a dose-related fashion among males. Also, there were increased incidences of fatty metamorphosis and focal cellular changes of the liver for females and chronic inflammatory change of the kidney in females. These non-neoplastic findings provide support for the Agency's decision to require a full 2 year chronic toxicity study in the Fischer 344 rat as detailed in the Reregistration Standard.

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3. Malathion - Sprague-Dawley Rat Dietary Feeding Carcinogenicity Study

Reference: Rucci, G., P.J. Becci and R.A. Parent. May 13, 1980. The evaluation of the chronic toxicity effects of Cythion administered in the diet to Sprague-Dawley rats for 24 consecutive months. Unpublished study (No. 5436) prepared by Food and Drug Research Laboratories, Inc., Waverly, NY for Agricultural Division, American Cyanamid Co., Princeton, NJ. Reviewed in Document #s: 002504, 004208).

Malathion (technical Cythion; purity 92.1%) was administered in the diet to groups of 50 male and 50 female Sprague-Dawley rats (from Blue Spruce Farms, NY) at doses of 100, 1000 or 5000 ppm per group for 24 months. Matched controls consisted of 50 untreated rats per sex. Surviving animals were killed at the end of the 24 month period (104 weeks).

The malathion Reregistration Standard states "this study was determined by the Agency to be unacceptable for use as either a chronic rat toxicity study or as a rat oncogenicity (sic) study. An independent reevaluation of the microscopic slides from this study is required in order to determine the acceptability of this study."

a. Consideration of Study Adequacy for Assessment of Carcinogenic Potential

This study was determined to be insufficient to provide definitive evidence on the carcinogenicity of malathion. A final review of this study in a Data Evaluation Report prepared by R.B. Jaeger (July 17, 1987) concluded this study should be classified as invalid. Many reasons were provided for the invalid classification, including: the summary tables do not distinguish between animals killed at term and animals found dead, killed moribund or accidental deaths; pathology slides were not read "blind", rather each pathologist had prior knowledge of the dose level administered; there is no indication of the numerical rating for the degree or severity of change observed by each pathologist; there were several pathologists involved which raises substantial concern for "consistency" and "uniformity", especially in light of several apparent discrepancies noted in the findings; animals which died, killed moribund or accidental deaths were not examined in a manner to preclude autolysis of tissue; animals in all groups suffered from substantial degrees of several illnesses, raising a question about good animal husbandry for this study; the substantial amount of geriatric changes in all groups makes it extremely difficult to separate or identify normal ageing processes from compound-related effects; kidney, pituitary, adrenals and thyroid weights were selectively screened and eliminated from the organ weight and organ-to-body weight comparisons if they were above or below pre-selected values; sufficient information for

examining biochemical and clinical effects in a chronic bioassay were not obtained; and, use of chloroform to euthanize animals at termination of the study is not presently a common practice as it is a suspect carcinogen and may induce potential adverse effects on its own. These points serve to illustrate the substantial faults in the study design, conduct and reporting of this study.

b. Discussion of Tumor Data

Despite the substantial problems with this study, a statistical analysis revealed some increased tumor incidences (memorandum C.J. Nelson, July 21, 1987). Uterus polyps in females had no significant trend, but both high and low dose groups were significantly different from controls by pair-wise comparison. There was a significant trend, but no pair-wise comparison difference for thyroid parafollicular cell (C-cell) malignant tumors in female rats. It was concluded by the Peer Review Committee however that there should not be much weight put upon these findings.

c. Non-Neoplastic Findings

Many chronic effects were noted in this study, some even at the low dose of 100 ppm. Statistical analysis (memorandum C.J. Nelson, July 21, 1987) revealed several significant effects which included: swollen liver and kidney glomerulosclerosis, prostate calcification, liver sinusoidal dilation, lymph node reticuloendothelial hyperplasia, pituitary cyst, and heart inflammation in male rats; kidney tubular casts, spleen extramedullary hematopoiesis, thymus cyst, uterus pyometra, kidney tubular dilation, and pancreas duct dilation in female rats. Though this study is unacceptable for a chronic toxicity study, these effects suggest concern for chronic non-neoplastic adverse effects induced by malathion.

4. Malathion - B6C3F1 Mouse Dietary Feeding Carcinogenicity Study

Reference: National Cancer Institute. 1978. Bioassay of malathion for possible carcinogenicity, CAS No. 121-75-5. Technical Report Series, No. 24, National Cancer Institute, Bethesda, MD. NCI-CG-TR-24. Assay performed at Gulf South Research Institute, New Iberia, LA. Report authored by M. Steinberg et al. at Tracor Jitco under NCI direction. Reviewed in Document # 000314.

Malathion (technical grade; purity $\geq$95%) was administered in the diet to groups of 50 male and 50 female B6C3F1 mice (from Charles River Breeding Laboratories, MA) at doses of 8000 or 16,000 ppm per group for 80 weeks. Animals were then observed for an additional 14 or 15 weeks. Matched controls consisted of groups of 10 untreated mice of each sex. Pooled controls consisted of the

matched controls combined with 40 untreated male and 40 untreated female mice from similar bioassays of four other test chemicals. These other pooled controls came from bioassays performed at the same laboratory and overlapped the malathion bioassay by at least 1 year. All surviving mice were killed at 94 to 95 weeks.

a. Discussion of Tumor Data

The NCI concluded that under the conditions of this study, there was "no clear evidence" of an association between malathion administration and tumor incidence. The NCI report noted a possible increased incidence of hepatocellular carcinoma in male mice (see Table 5). Statistical treatment showed a dose-related trend ($p = 0.019$) when neoplastic nodules and hepatocellular carcinoma were combined and compared to pooled controls. The direct comparison between the high-dose group and the pooled control group for combined nodules and carcinoma revealed a significant difference ($p = 0.031$ Fisher's Exact Test); however, the NCI employed as its criteria of significance $p = 0.025$, based on Bonferroni adjustments, and therefore did not consider this a positive finding.

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Table 5: Malathion B6C3F1 Mouse Study - Liver Findings from NCI Evaluation

Males	C1	C2	Low Dose	High Dose
Hepatocellular Carcinoma	2/10 (20%)	5/49 (10.2%)	7/48 (14.6%)	11/49 (22.5%)
Neoplastic Nodules & Hepatocellular Carcinoma	----	8/49 (16.3%)	7/48 (14.6%)	17/49 (34.7%)

C1 = Matched Controls
C2 = Pooled Controls
Number of Lesions/Number of tissues examined (Incidence %)

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The Reregistration Standard states "because of study design flaws and the questionable liver findings (i.e. dose-related trend ($p = 0.019$) and increased incidence at hepatocellular carcinomas at the high dose ($p = 0.031$), another study in mice is required." Further considerations by Toxicology Branch I/Health Effects Division and this Peer Review Committee also raise doubt as to how definitive this study is to make a clear conclusion about the carcinogenicity of malathion. Although the NCI did not consider the pair-wise comparison significant based on Bonferroni adjustments, the significance value ($p = 0.031$) is still at a level

the Agency considers significant ($p \leq 0.05$). However, from the NCI evaluation, there is not enough data presented (e.g. individual animal data, information about the "pooled" controls) to allow an independent statistical treatment of the data by statisticians supporting the Peer Review Committee. This makes it difficult for the Peer Review Committee to make an independent decision based on their own analyses of the data. Therefore, it is not entirely clear that the suggestive evidence of male hepatocellular carcinoma and combined neoplastic nodules/hepatocellular carcinoma can be totally dismissed.

b. Considerations of Study Adequacy for Assessment of Carcinogenic Potential

Other considerations provide even greater doubt about the adequacy of this study to make definitive conclusions regarding the carcinogenicity of malathion. 1) This study was performed before the Good Laboratory Practice (GLP) standards were in place. A significant deviation from current GLPs is the length of dosing in this study where mice received malathion for 80 weeks instead of the entire study period. 2) The number of concurrent control animals (10) is a very small group; this makes comparative analyses difficult when there is not a dramatic difference between tumor incidences. It cannot be concluded with certainty that if a larger concurrent control size had been used, the incidence of spontaneous tumors would rise proportionately. 3) Without detailed information about the pooled control animals, the appropriateness of using these animals as "concurrent" controls is unclear. 4) It was noted that the historical control incidence data for hepatocellular carcinoma in this strain of mouse often is higher than that observed in the high-dose group seen in this study. 5) The Registrant points out that the dose levels employed in this study (8000 and 16,000 ppm) exceed the OPP accepted upper limit dose of 1.0 g/kg/day in mouse carcinogenicity studies. 6) The NCI report lists a number of signs of disease appearing "with increasing frequency in dosed animals", especially at weeks 72 and beyond to the end of the study. This raises questions about the general health of animals during a crucial period of the study.

Since there were many uncertainties about the conduct of this study and the questionable liver findings, the Peer Review Committee endorsed the requirement for an additional mouse carcinogenicity study with malathion to help clarify any possible carcinogenic potential by malathion.

5. Malaoxon - Fischer 344 Rat Dietary Feeding Carcinogenicity Study

Reference: National Cancer Institute. 1979. Bioassay of malaoxon for possible carcinogenicity, CAS No. 1634-88-2. Technical Report Series, No. 135, National Cancer Institute, Bethesda, MD. NCI-CG-

TR-135. Assay performed at Gulf South Research Institute, New Iberia, LA. Report authored by C.R. Angel et al. at Tracor Jitco under NCI direction. Reviewed in Document # 000314.

Malaoxon (synthesized by testing laboratory, purity >95%) was administered in the diet to groups of 50 male and 50 female Fischer 344 rats (from NCI Frederick Cancer Research Center, MD) at doses of 500 or 1000 ppm per group for 103 weeks. Animals were then observed for up to an additional 2 weeks. Matched controls consisted of 50 untreated rats per sex. All surviving rats were killed at 103 to 105 weeks.

a. Discussion of Tumor Data

The original NCI review concluded that under the conditions of this study, malaoxon was not carcinogenic in Fischer 344 rats. The review did note a possible increase in thyroid C-cell adenomas or carcinomas in female rats at the high dose. However, this positive finding for C-cell tumors was precluded by "historical" control data. The review states "the historical record of this laboratory shows an incidence of female F344 rats with C-cell adenomas or carcinomas of 16/223 (7%), compared with 0/50 in the control group, 1/49 (2%) in the low-dose group and 5/47 (11%) in the high-dose group of this study. This indicates that the incidence of C-cell tumors of the thyroid in female rats of the present study is comparable to that usually seen in control animals." The NCI report also revealed an increased incidence of benign mammary gland tumors in low-dose females ($p = 0.026$). However, this increase was not considered to be significant as the NCI employed as its criteria of significance $p = 0.025$, based on Bonferroni adjustments, and therefore did not consider this a positive finding. The original NCI report also noted an increase in the incidence of adrenal gland pheochromocytoma in males, but reported these increases were not statistically significant.

The NTP reevaluated tissues from these organs (thyroid gland, adrenal gland) as potential suggestive targets. The NTP reevaluation revealed one difference from the original NCI review. The NTP concluded that there was equivocal evidence of carcinogenicity for male and female F344 rats based on findings for C-cell neoplasms of the thyroid gland (Huff et al., 1985). NTP examinations of the major sites of potential targets are shown in Table 6. In particular, the NTP reevaluation resulted in an increase in incidence of C-cell tumors in females that was significant at the high-dose ($p = 0.05$) and yielded a dose-related trend. For males, the incidence of C-cell hyperplasia was not as evident as in the original NCI review, but the NTP revealed a positive finding for adenomas and carcinomas in high-dose males ($p \leq 0.05$). The NTP reevaluation also showed an increase in the incidence of mammary gland adenomas in low-dose females, but this increase was dismissed as related to malaoxon administration as the

increase was not seen at the high dose and the incidence in the concurrent controls was unusually low. The subsequent NTP reevaluation resulted in a considerable revision in the incidence of pheochromocytoma; however, the NTP did not indicate any statistically significant findings for this tumor.

The NTP provided a detailed description of their reasoning for considering the equivocal evidence for C-cell neoplasms of the thyroid gland (Huff et al., 1985). Arguments for an associative effect by malaoxon, from the publication, are: (i) dose-response trends in both sexes, (ii) the incidences in the high-dose groups were increased, albeit marginally, in comparison to concurrent controls, (iii) the incidences exceed the historical rates observed in this species (male F344 rats, 196/2230, 8.8%; female F344 rats, 190/2265, 8.4%), and (iv) six carcinomas were found in the high-dose groups, compared with one in the controls. The arguments against this being a malaoxon related response are: (i) no corresponding increases were seen for hyperplasia (see Table 6 below), (ii) the neoplasms were microscopic in size and morphologically identical to naturally occurring tumors, (iii) no supporting effects were observed in Study II of malathion in F344 rats or in the Study I of malathion in Osborne-Mendel rats, both at higher doses, and (iv) the incidence in the concurrent control group was somewhat lower than the rates observed in Study II of malathion and the mean historic control.

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Table 6: Malaoxon Fischer 344 Rat Study - Findings of NTP Reevaluation

	Sex	Control	Low Dose	High Dose
Thyroid C-cell hyperplasia	M	8/49 (16%)	11/45 (24%)	8/49 (16%)
	F	24/48 (50%)	24/48 (50%)	25/48 (52%)
Thyroid C-cell adenoma & carcinoma	M	3/49 (6%)	3/45 (7%)	10/49 (20%)
	F	4/48 (8%)	7/48 (15%)	11/48 (23%)*
Adrenal Pheochromocytoma	M	5/50 (10%)	6/50 (12%)	10/49 (20.4%)
Mammary gland adenoma	F	2/50 (4%)	9/50 (18%)	1/50 (2%)

Number of Lesions/Number of tissues examined (Incidence %)
* may be 12/48 (25%)

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The Reregistration Standard for malathion requires that the Registrant perform an additional Fischer 344 rat study using

malaoxon. The purpose of this additional study is to clarify the carcinogenic potential of malaoxon and provide additional needed data on the effects of malaoxon on cholinesterase inhibition. Further considerations by Toxicology Branch I/Health Effects Division and this Peer Review Committee raise doubt as to how definitive this study is to make a clear conclusion about the carcinogenicity of malaoxon. Again, as in the Osborne-Mendel rat study, the NTP does not provide detailed data from which to perform independent statistical analyses. Therefore, it is not entirely clear that the suggestive evidence of male adrenal gland pheochromocytomas and of female mammary gland adenomas at the low dose can be totally dismissed.

The Peer Review Committee agrees with the NTP language regarding the equivocal evidence for the C-cell neoplasms of the thyroid gland; i.e. "equivocal evidence of carcinogenicity is demonstrated by studies that are interpreted as showing a chemically-related marginal increase of neoplasms." The Peer Review Committee made several comments on the detailed reasoning the NTP provided on the equivocal classification. 1) While the incidences of C-cell neoplasms are above the mean historical values provided by the NTP, it is not clear what the range of values were from the individual studies that made up the historical database. This may have some bearing on the significance of the increased thyroid findings. 2) It was noted that the thyroid C-cell hyperplasia rate was not significantly elevated over control. If thyroid neoplasms are a result of a progression from hyperplasia to adenoma to carcinoma, then this lack of an effect appears important in this regard. It also may be a reflection of the study design. 3) It was considered that the elevated number of carcinomas observed was a significant contribution to the possible chemical induced effect by malaoxon. 4) As regards to the possible low concurrent control group incidence, it was noted that the NTP incidence was similar to the original NCI incidence. 5) There was question as to whether it can be definitively concluded that there were no supporting effects seen in the two malathion rat carcinogenicity studies.

Therefore, due to the uncertainty of the total findings in this malaoxon study, the Peer Review Committee reaffirms the Reregistration Standard requirement for an additional rat carcinogenicity study using malaoxon.

b. <u>Consideration of Study Adequacy for Assessment of Carcinogenic Potential</u>

Another consideration by the Peer Review Committee and Toxicology Branch I provides support for requiring an additional rat study with malaoxon. The percent survival at week 90 of the study for males and females, respectively, was 80% and 82% for controls, 82% and 90% for the low dose group, and 64% and 80% for the high dose group. For the male animals, there appears to be a

dose-dependent increase in mortality that raises the issue of the actual number of animals at risk at the end of the study. It is not clear if the NTP took the higher mortality into account in their deliberations although the NTP mentioned in their reevaluation that sufficient numbers of rats of each sex were at risk for the development of late appearing tumors.

c. Non-Neoplastic Findings

The NTP reports that in this study forestomach ulcers were observed at increased incidences in male and female rats (males: 3/47, 6%, control; 7/48, 14%, low dose; 9/48, 18%, high dose; and, females: 0/49, 0%, control; 1/48, 2%, low dose; 3/48, 6%, high dose). This is similar to the findings evaluated by the NTP in the malathion Fischer 344 rat study.

6. Malaoxon - B6C3F1 Mouse Dietary Feeding Carcinogenicity Study

Reference: National Cancer Institute. 1979. Bioassay of malaoxon for possible carcinogenicity, CAS No. 1634-88-2. Technical Report Series, No. 135, National Cancer Institute, Bethesda, MD. NCI-CG-TR-135. Assay performed at Gulf South Research Institute, New Iberia, LA. Report authored by C.R. Angel et al. at Tracor Jitco under NCI direction. Reviewed in Document # 000314.

Malaoxon (synthesized by testing laboratory, purity >95%) was administered in the diet to groups of 50 male and 50 female B6C3F1 mice (from NCI Frederick Cancer Research Center, MD) at doses of 500 or 1000 ppm per group for 103 weeks. Animals were then observed for up to an additional 2 weeks. Matched controls consisted of 50 untreated mice per sex. All surviving mice were killed at 103 to 105 weeks.

The NCI report concluded that under the conditions of this study, malaoxon was not carcinogenic in the B6C3F1 mouse. The OPP review of this study concurred with the NCI in this opinion and the malathion Reregistration Standard does not call for additional testing of malaoxon in the mouse.

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E. Additional Toxicology Data on Malathion:

1. Acute Toxicity

Technical malathion is mildly toxic on an acute oral (Category III), dermal (Category III) and inhalation (Category III) basis. Technical malathion is only mildly irritating to the eye of rabbit (Category III) and slightly irritating after dermal exposure to rabbit (Category IV). Technical malathion is nonsensitizing by dermal application. No data are available on the acute delayed neurotoxicity of malathion in the hen and, since malathion is an organophosphate, this study is required.

2. Metabolism

According to the Malathion Reregistration Standard issued in February, 1988, there are data gaps in the chronic toxicology data base which includes a data gap for metabolism studies. While the OPP does not have submitted acceptable studies concerning malathion metabolism, malaoxon is considered to be a metabolite of malathion. However, it is not clear how much malathion is metabolized to malaoxon. Since malaoxon is a metabolite of malathion, and may be responsible for some or all malathion toxic effects, malaoxon was also examined for carcinogenicity in long term bioassays.

3. Mutagenicity

According to the Malathion Reregistration Standard issued in February, 1988, there are no data available on the mutagenic potential of malathion. Studies are required in all of the following mutagenicity test areas: gene mutation, structural chromosomal aberrations, and other genotoxic effects. While there are no acceptable studies submitted to the OPP, there are many open literature articles concerning mutagenicity testing with malathion and malaoxon. Tables 7 and 8 present a listing of many of these tests (this is not prepared as an exhaustive or critical review).

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Table 7. Open Literature Mutagenicity Studies on Malathion

Gene Mutation Category

Salmonella assay
Haworth et al., Environ Mutagen 5 (Suppl 1): 3-142, 1983
Moriya et al., Mutat Res 116: 185-216, 1983
Waters et al., Basic Life Sci 21: 275-326, 1982
Other references
Results: all Negative

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E. coli reverse mutation (WP2; WP2 uvr A)
Brusick et al., Mutat Res 76: 169-190, 1980
Waters et al., Basic Life Sci 21: 275-326, 1982
Moriya et al., Mutat Res 116: 185-216, 1983
Results: all Negative

Mouse lymphoma assay
NTP, 1988 Annual Plan
Result: Equivocal

Drosophila sex-linked recessive lethal assay
Waters et al., Basic Life Sci 21: 275-326, 1982
Velazquez et al., Environ Mutagen 9: 343-348, 1987
Results: all Negative

Structural Chromosomal Aberrations Category

In vitro mammalian cell aberrations
Galloway et al., Environ Mol Mutagen 10 (Suppl 10): 1-175, 1987
Result: CHO cells, Negative w/o activation, Positive w/act.
Ishidate et al., Mutat Res 195: 151-213, 1988
Results: CHL cells, Positive ± activation
human lymphocytes, Positive w/o activation
human hematopoietic B411-4 cells, Negative w/o act.

In vivo mammalian aberrations - bone marrow
Dulout et al., Mutat Res 122: 163-167, 1983
Result: Positive, one i.p. dose Balb/c mouse
Degraeve and Moustschen, Environ Res 34: 170-174, 1984
Result: Negative, one i.p. dose Q strain mouse
Degraeve et al., Arch Toxicol 56: 66-67, 1984
Result: Negative, 7 weeks drinking water, a low dose (8 ppm)
Dzwonkowska and Hubner, Arch Toxicol 58: 152-156, 1986
Result: weak Positive, one i.p. dose Syrian hamster
Salvadori et al., Mutat Res 204: 283-287, 1988
Result: one dose, Negative; multiple doses (5 days/2 weeks), Positive

Mouse micronucleus
Dulout et al., Mutat Res 105: 413-416, 1982
Result: Positive, cutaneous exposure; weak Positive, i.p.

In vivo human - acute malathion intoxication
van Bao et al., Humangenetik 24: 33-57, 1974
Result: increase in breaks in lymphocytes

In vivo mammalian aberrations - germ cells
Degraeve and Moutschen, Environ Res 34: 170-174, 1984
Result: Negative, spermatogonia, one i.p. dose Q strain mouse
Degraeve et al., Arch Toxicol 56: 66-67, 1984
Result: Negative, spermatogonia and 1^{o} spermatocytes, 7 weeks drinking water, 8 ppm, Q strain mouse

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Salvadori et al., Mutat Res 204: 283-287, 1988
Result: one dose, 1° spermatocytes, Negative
multiple doses, 1° spermatocytes, Positive

Dominant lethal assay - mouse
3 reported Negative studies, but problems with each study

Note: Krause et al., Bull Environ Contam Toxicol 15: 458-462, 1976
Result: found slight damage to testicular tissues, but recovered, indicate malathion can reach these tissues

Other Genotoxic Effects Category

In vitro mammalian cells - SCE
Galloway et al., Environ Mol Mutagen 10 (Suppl 10): 1-175, 1987
Result: Positive ± activation, CHO cells
Nicholas et al., Mutat Res 67: 167-172, 1979
Result: Positive w/o activation, human fetal lung fibroblasts
Nishio and Uyeki, J Toxicol Environ Health 8: 939-946, 1981
Result: Positive w/o activation, CHO cells
Chen et al., Mutat Res 88: 307-316, 1981
Result: weak, but dose response Positive w/o act., V79 cells
Chen et al., Environ Mutagen 4: 621-624, 1982
Result: weak, but dose response Positive with act., V79 cells
Sobti et al., Mutat Res 102: 89-102, 1982
Result: Positive ± activation, human lymphoid cells (LAZ-007)

UDS in WI-38 cells
Waters et al., Basic Life Sci 21: 275-326, 1982
Result: Negative

Mitotic recombination in Saccharomyces
Waters et al., Basic Life Sci 21: 275-326, 1982
Result: Negative

Differential toxicity in DNA repair deficient strains of E. coli and B. subtilis
Waters et al., Basic Life Sci 21: 275-326, 1982
Result: Negative

Other:
Griffin and Hill, Mutat Res 52: 161-169, 1978
Result: induced in vitro breakage of plasmid DNA at a slow rate, but significantly greater than control rate

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Table 8. Open Literature Mutagenicity Studies on Malaoxon

Gene Mutation Category

Salmonella assay
Zeiger et al., Environ Molec Mutagen 11 (Suppl 12): 1-158, 1988
Result: Negative

Mouse lymphoma assay
Tennant et al., Science 236: 933-941, 1987
Result: Positive without activation

Structural Chromosomal Aberrations Category

Aberrations/CHO cells
Ivett et al., Environ Molec Mutagen 14: 165-187, 1989
Result: Negative

Other Genotoxic Effects Category

SCE/CHO cells
Ivett et al., Environ Molec Mutagen 14: 165-187, 1989
Result: Positive ± activation, but weak with activation
Nishio and Uyeki, J Toxicol Environ Health 8: 939-946, 1981
Results: Positive without activation (slightly greater than malathion

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These mutagenicity data indicate that malathion appears to have genetic activity. It is positive in all the available sister chromatid exchange assays. Providing even greater support to a mutagenicity concern is the appearance of positives in both in vitro and in vivo cytogenetic assays, in both somatic and germ cells. This information provides support for a possible genetic component in the weight of evidence consideration for carcinogenicity as well as for a possible heritable mutagenicity concern. It is important that the malathion Reregistration Standard requirements be followed. The limited amount of available malaoxon mutagenicity data appear consistent with the malathion data.

4. Developmental and Reproductive Effects

A developmental study was performed with New Zealand rabbits. Twenty female rabbits per dose group were exposed via gastric intubation to single daily doses of vehicle (corn oil) or malathion on days 6 to 18 gestation. Dose levels used were 25, 50 and 100 mg/kg body weight per exposure. Animals were observed between days 0 to 20 of gestation. No adverse developmental effects were seen. Due to decreased body weight gain at 50 mg/kg/day and increases in

mean percent of resorptions at the top two dose groups, the developmental No Observable Effect Level (NOEL) is 25 mg/kg/day and the maternal NOEL is also 25 mg/kg/day.

The Reregistration Standard for malathion states that a rat developmental toxicity study is required to support registration of products containing malathion. A reproduction study in the rat is also required to be performed.

5. Structure-Activity Correlations

There was not a great deal of discussion regarding possible structure-activity correlations with malathion. It appears that as a general class organophosphates are not consistent in their actions, probably due to their differences in toxicity, metabolism, distribution, etc. However, it was emphasized that malaoxon is a major metabolite of malathion and that several of the suggestive findings in the malathion rat carcinogenicity studies are apparent in the malaoxon rat carcinogenicity study. These included lesions in the thyroid and adrenal glands. Furthermore, some non-neoplastic findings were comparable, especially the occurrence of stomach ulceration. Finally, the genetic toxicity data appear to be consistent between malathion and malaoxon, at least based on the limited amount of data available for malaoxon.

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F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Malathion to be of importance in a weight-of-the-evidence determination of carcinogenic potential.

1) The major consideration of the Peer Review Committee was the inadequacy of the available studies to make definitive determinations on the carcinogenicity of malathion and malaoxon. There were many issues raised concerning the adequacy of each study from which a firm conclusion regarding carcinogenicity could not be reached.

2) In addition to the equivocal evidence for carcinogenicity in the malaoxon Fischer 344 rat study for C-cell neoplasms of the thyroid gland for males and females, there are other suggestions of carcinogenic responses in 5 of the 6 studies considered:

Osborne-Mendel rat: (malathion)	neoplasms of thyroid gland, male and female pheochromocytoma of adrenal gland, male
Fischer 344 rat (malathion)	: pheochromocytoma of adrenal gland, male leukemia, male
Sprague-Dawley rat: (malathion)	neoplasms of thyroid gland, female uterus polyps, female
B6C3F1 mouse (malathion)	: neoplastic nodules/hepatocellular carcinoma, male
Fischer 344 rat (malaoxon)	: equivocal evidence for C-cell neoplasms of the thyroid gland, male and female pheochromocytoma of adrenal gland, male mammary gland adenomas, female

3) While the NTP does not attribute the appearance of the different tumors to malathion or malaoxon administration (outside of the equivocal evidence for C-cell neoplasms in the malaoxon rat study), in many instances, the same tumor types appear in different studies (see point above for specifics). Also, in several instances, more than one tumor type was suggested by the study.

4) The mutagenicity data indicate that malathion appears to have genetic activity. This information provides support for a possible genetic component in the weight of evidence consideration for carcinogenicity. The limited amount of available malaoxon mutagenicity data appear consistent with the malathion data.

5) The NTP has indicated in its memorandum to J. Moore (from E. McConnell) that the NTP is considering a further study of malathion using current "state-of-the-art" methods. There has been no further information on this intention. Furthermore, the NTP's

Board of Scientific Counselors has recommended that there is need for a state of art carcinogenesis study for malathion (NTP Fiscal Year 1986 Annual Plan, NTP Publication No. NTP-86-086). This indicates that although they have no reason to believe malathion is carcinogenic, there is a perceived need for a state-of-the-art carcinogenicity study for malathion. This supports the Reregistration Standard's requirements for further carcinogenicity studies as well as the requirement for combining the carcinogenicity aspects to the required rat chronic study for malathion.

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G. Classification of Carcinogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

The Peer Review Committee unanimously agreed to classify malathion as a Group D Carcinogen; that is, malathion is not classifiable as to human carcinogenicity. The Committee could not completely endorse the NTP conclusion that there was no clear evidence of carcinogenicity due to malathion or malaoxon administration in most of these studies (the NTP concluded equivocal evidence in the malaoxon rat study). The Peer Review Committee decision was based on the inadequacy of the available studies to make definitive determinations on the carcinogenicity of malathion and malaoxon. There were many issues raised concerning the adequacy of each study from which a firm conclusion could not be obtained.

In addition, while there may have been doubts about the significance of each tumor type in each of the individual studies, there was the suggestive appearance of similar tumors (e.g. neoplasms of the thyroid gland and of the adrenal gland) and of multiple tumors in more than one study. Also, there was some evidence from mutagenicity studies that a genetic component for malathion and malaoxon is possible. These points provided weight to the evidence of possible carcinogenic effects that could not be totally dismissed.

The Committee reaffirmed the requirements of the Malathion Reregistration Standard that requires the Registrant to perform an additional mouse carcinogenicity study with malathion and an additional rat carcinogenicity study with malaoxon. The Committee also determined that the Reregistration Standard recommendation to perform a carcinogenicity study in combination with a rat chronic study on malathion be made into a requirement. It is believed these studies using current standards will provide adequate evidence for a definitive decision regarding the carcinogenicity classification for malathion.

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